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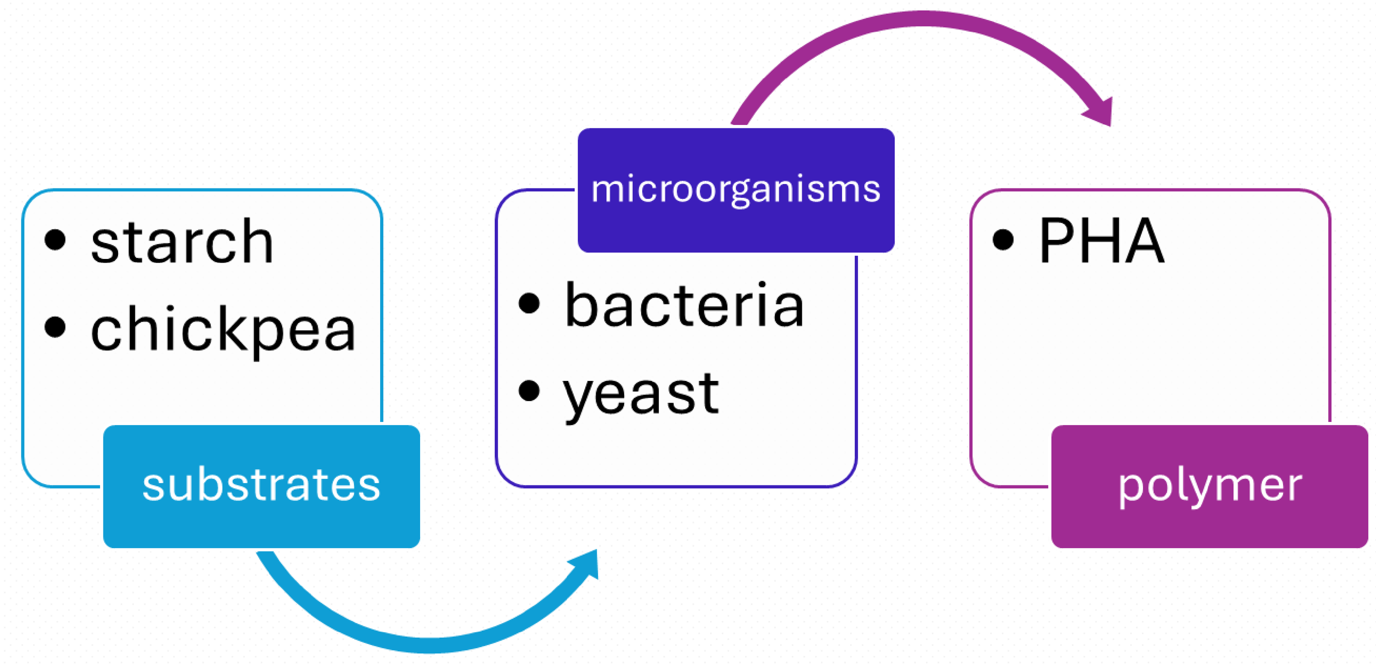
Isolation and identification of microorganisms from waste biomass and their application in the production of polyhydroxyalkanoates

Dora Bramberger[1], Martina Miloloža[1], Krešimir Stublić[2], Matija Cvetnić[1], Vesna Ocelić Bulatović[1], Šime Ukić[1], Tomislav Bolanča[1], Marinko Markić[1], Lidija Furač[1], Jasmina Ranilović[3], Dajana Kučić Grgić[1]

[1]University of Zagreb Faculty of Chemical Engineering and Technology, Trg Marka Marulića 19, 10000 Zagreb
[2]AQUA V.M.V. d.o.o., Ulica kralja Zvonimira 98, 10000 Zagreb
[3]Podravka d.d., Ante Starčevića 32, 48000 Koprivnica

INTRODUCTION

- Conventional plastics - non-degradable and cause significant environmental problems
- Polyhydroxyalkanoates (PHAs) are a viable alternative
- PHA synthesis: microorganisms convert CARBOHYDRATES and PROTEINS into SUGAR for PHA synthesis
- PHA applications: packaging, implants, coatings, 3D printing, drug delivery and tissue engineering



MATERIALS & METHODS

Substrates

STEP 1

Isolation of microorganisms

- **Bacteria**
- **Fungi**
  - Yeast

STEP 2

Identification of microorganisms

Bacteria:
- Gram staining
- Sudan black staining
- KOH test
- Biochemical tests: catalase, oxidase and nitrate-reductase tests
- API Strips

Yeast:
- Sudan black staining
- API 20 C aux

STEP 3

Solid – state fermentation (SSF) and extraction and purification of PHA

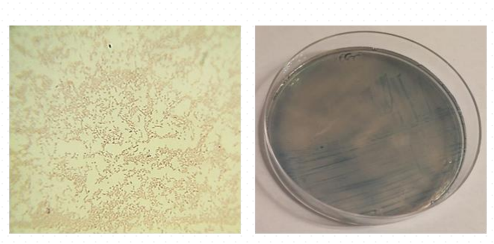
- The SSF was performed for 7 days at room temperature
- The SSF was carried out for:
  - starch
  - chickpea 1
  - chickpea 2
  - mixture of starch, chickpea 1 and chickpea 2
  - sterilised mixture of starch, chickpea 1 and chickpea 2
- to 100 g of each substrate and mixture, 10 mL of suspension of mixed culture was added
- to 100 g of sterilised mixture of starch, chickpea 1 and chickpea 2, 10 mL of *Cupriavidus necator* suspension was added
- After SSF, the substrates were centrifuged and dry cell weight (DCW) was determined
- Extraction and purification of PHA was performed with chloroform and 4% sodium hypochlorite

STEP 4

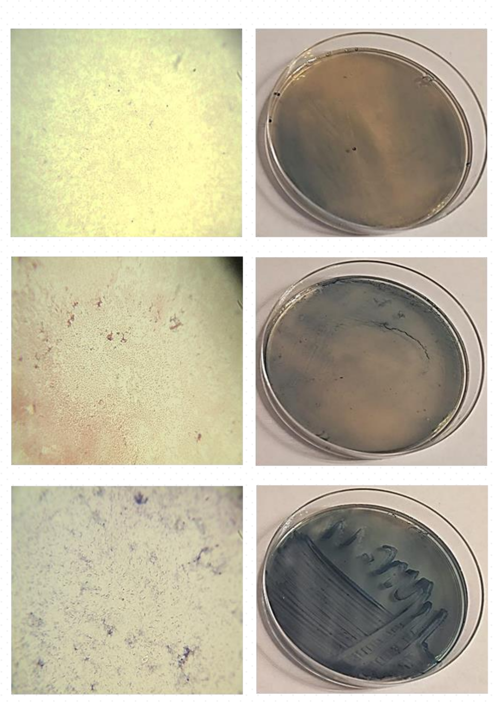
Characterization od PHA

- **FTIR – ATR spectroscopy**
  - wavelenght range: 4000 – 650 cm^{-1}
- **DSC**
  - 5 – 7 mg of samples
  - 5 cycles of heating and cooling
- **TGA**
  - 5 – 7 mg of samples
  - 40 – 700 ^{o}C

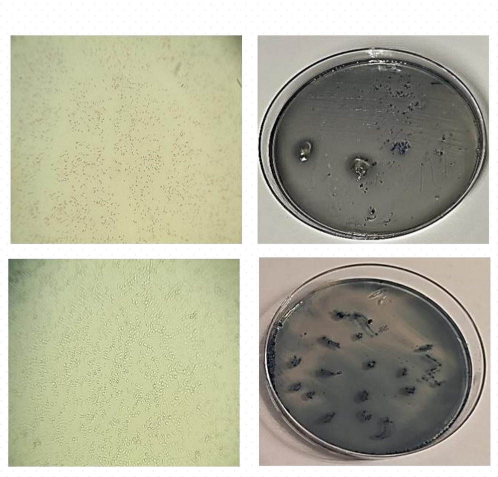
RESULTS



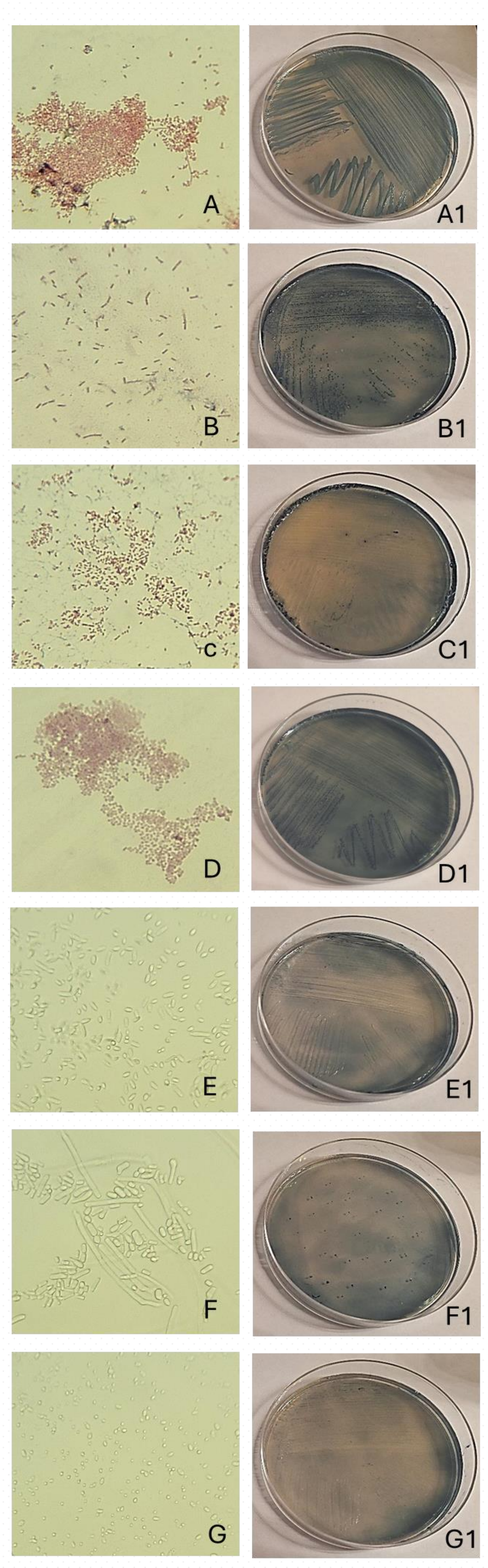
Microphotograph of Gram staining (A) and photograph of Sudan Black staining (A1) of bacterium *Cupriavidus necator*.



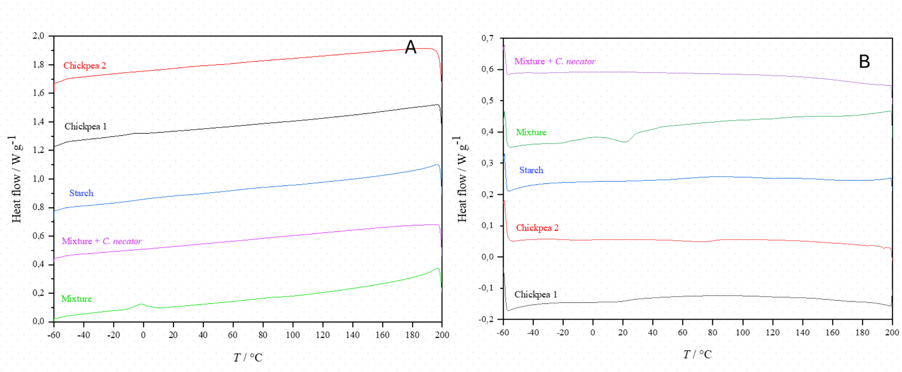
Microphotographs of Gram staining and photographs of Sudan Black staining of bacteria *Brevibacillus* sp. (A, A1), *Empedobacter brevis* (B, B1) and *Aneurinibacillus aneurinilyticus* (C, C1), respectively, isolated from chickpea 1.



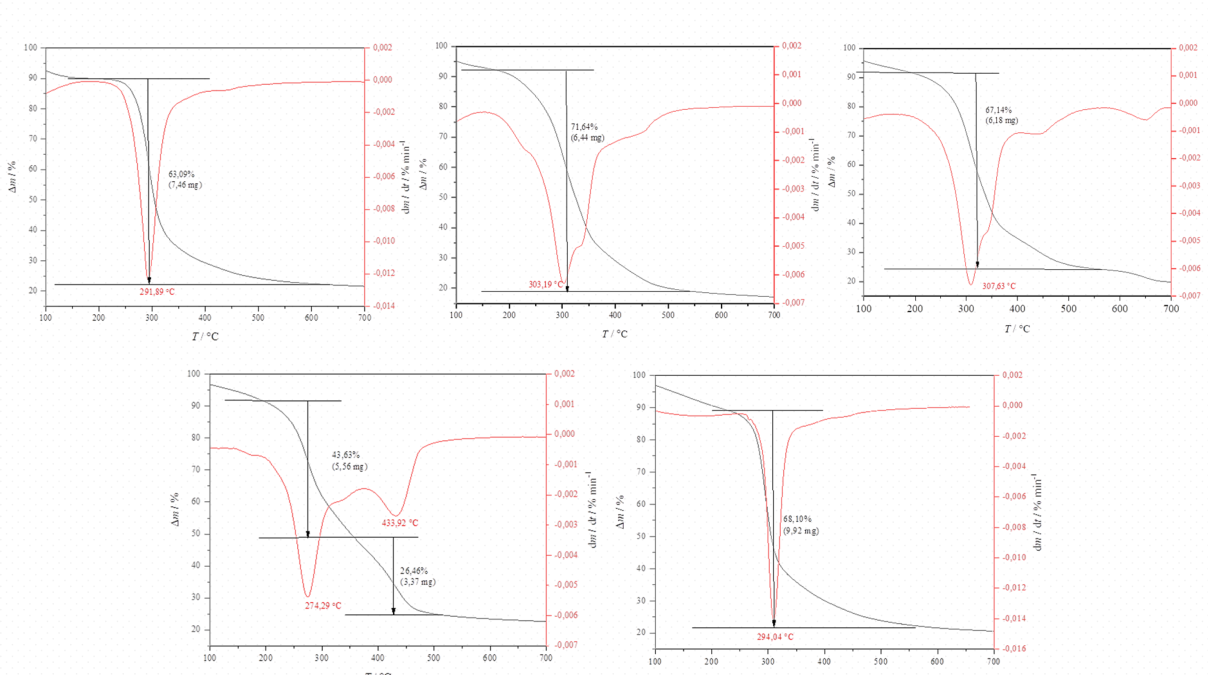
Microphotographs of Gram staining and photographs of Sudan Black staining of bacterium *Micrococcus* spp. (A, A1), and yeast *Trichosporon asahii* (B, B1), respectively, isolated from chickpea 2.



Microphotographs of Gram staining and photographs of Sudan Black staining of bacteria *Leukonostoc sp.* (A, A1), *Bacillus licheniformis* (B, B1), *Citrobacter freundii* (C, C1), *Staphilococcus lentus* (D, D1), and yeast *Cryptococcus humicola* (E, E1), *Geotrichum klebahnii* (F, F1), *Candida krusei* (G, G1), respectively, isolated from starch.



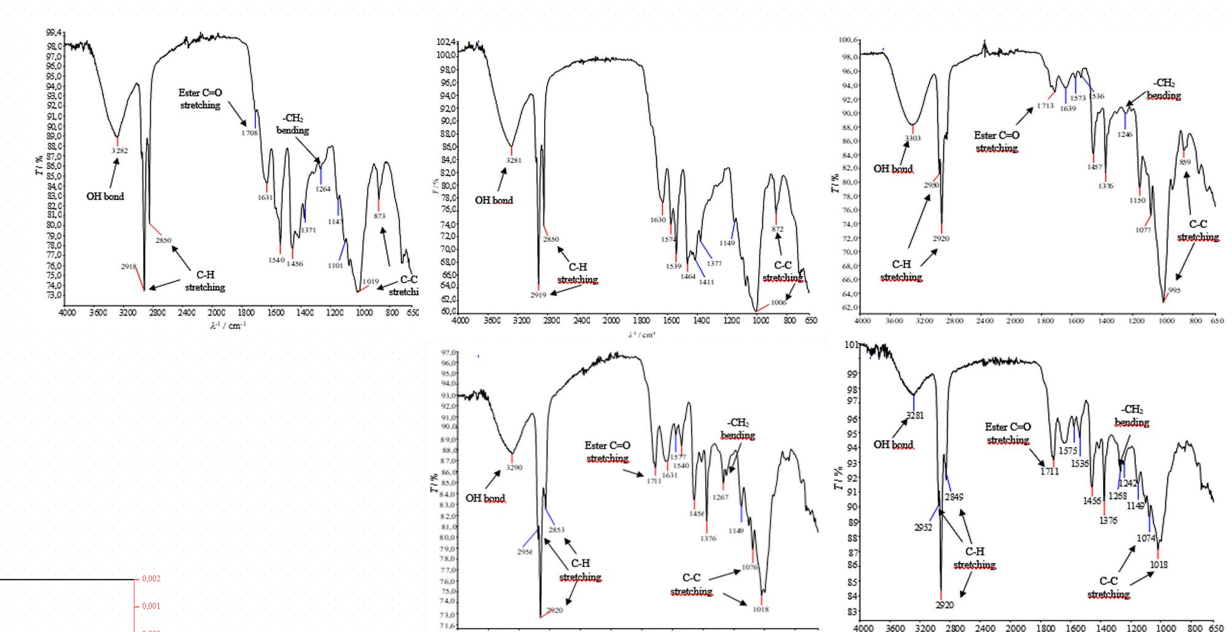
DSC thermograms of cooling (A) and heating (B) of PHA obtained by SSF of different substrates after 7 days.



TGA/DTG curves for PHA obtained by SSF of starch (A), chickpea 1 (B), chickpea 2 (C), mixture of substrates(D), and mixture of substrates with *Cupriaviuds necator* (D) after 7 days.

Accumulation of PHA obtained by 5 substrates after 7 days of SSF.

Sample	PHA accumulation / %
Chickpea 1	5.42
Chickpea 2	**13.81**
Starch	5.29
Mixture	4.09
Mixture + C. necator	6.30



FTIR spectra of PHA obtained by SSF of chickpea 1 (A), chickpea 2 (B), starch (C), mixture (D), and mixture with *Cupriavidus necator* (E) after 7 days.

Characteristic funcional groups of PHA obtained by FTIR-ATR spectroscopy.

Sample	Wavenumbers / cm^{-1} -OH bond	C-H stretching	C=O stretching	$-CH_2$ bending	C-C stretching
Chickpea 1	3282	2918	1708	1264	1019
		2850			873
Chickpea 2	3281	2919	/	/	1006
		2850			872
Starch	3303	2950	1713	1246	995
		2920			859
Mixture	3290	2956	1711	1267	1076
		2920			1018
		2853			/
Mixture + *C. necator*	3281	2952	1711	1268	1074
		2920			1018
		2849			/

CONCLUSION

- 8 bacterial strains and 4 yeasts capable of PHA production were isolated from starch and chickpeas.
- The highest PHA accumulation (13.81 %) was obtained after SSF of chickpeas with the addition of bean residues.
- FTIR spectroscopy results show broad peaks around 3300 cm^{-1} indicating -OH binding of moisture, three peaks in the range of 2900 - 2800 cm^{-1} indicating $-CH_3$ and $-CH_2$ groups, and peaks around 1720 cm^{-1} indicating the absorption band of ester carbonyl groups characteristic of PHB.

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